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MICROBIOLOGICAL SAFETY EQUIPMENT[1]

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ABSTRACT. A program for biological safety in the animal laboratory must consist of the firm establishment of clearly stated principles, the education of all personnel in safety, the immunization of personnel where applicable and the use of properly designed safety equipment. Many laboratory infections are preventable by the judicious use of such specially designed equipment. This is exemplified by the procedures and equipment, described in this paper, which have been proven by actual practice over many years of tests with highly communicable disease agents.

The most important aspect of the problem of biological safety for infectious disease laboratories is the prevention of infection in laboratory personnel. Philosophically less important, but still of great practical significance, is the problem of also preventing laboratory acquired infections (cross-infection) in the animals used for experimentation. The need for minimizing animal cross-infection is best indicated by its prejudicial effect on experimental results and the infectious hazard presented to the handler of infected animals (Kirchheimer, Jemski and Phillips, 1961).

The simplest and the surest method to achieve this "prevention" is the prohibiting of personnel from working with certain agents infectious for man or animals. This principle actually has been enforced in some laboratories wherein work with certain disease organisms is not permitted. The general acceptance of this concept obviously would soon lead to a general ignorance of human and animal disease problems. A much more reasonable practice, therefore, is one in which the use of special techniques or equipment provides and maintains safe working conditions in the infectious disease unit. The absolute necessity of such techniques can be exemplified by the conceivable isolation of a virus as the etiological agent of human carcinomas. In this situation there undoubtedly would be a rising emphasis on the moral and legal obligation of laboratory chiefs to provide protection for all personnel associated with any work with this "cancer virus." Actually, these obligations have always resided in a supervisory position but in a great many instances have been tacitly ignored on the basis that occupational illness was inherent in the profession. The fact, however, is that many laboratory infections are preventable by the judicious use of specially designed equipment (Wedum, *et al.*, 1956a) and by the conscientious application of good techniques in biological procedures that are potentially hazardous (Smadel, 1951; Fish and Spendlove, 1950). The practical worth of these concepts was pointed out at the 1960 meeting of the Animal Care Panel in a report from our laboratory (Jemski, 1960). This report showed that not one case of illness in either our animal handler

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or technical personnel occurred in over 200,000 exposure-risk man hours even though the work involved direct handling of animals exposed to aerosols of highly infectious organisms. This past year's work has resulted in an additional 15,000 man hours of safe work.

Most of the publications relating to the frequency, the causes, and the elimination of laboratory acquired infections have led to the conclusion that the source of infection is generally within a few inches of the worker's face and that inclosure and ventilation are the major factors in eliminating laboratory infections. It, therefore, is apparent that the basic principle involved in the design and use of microbiological safety equipment is in limiting or controlling intimate or casual contact with the infectious agents. In the previous report from our laboratory, this principle was illustrated by the individual maintenance of experimentally infected animals in sealed cages ventilated with filtered air pulled through the cage. Such equipment shields animal from animal and handler from animal. These design features also typify the bacteriological safety cabinet which is the most important item of equipment in the prevention of laboratory acquired infections (Wedum, 1953). The ventilated cabinet is a device which not only provides suitable table top area for the performance of microbiological operations but also provides glass shielding for the worker.

A variety of ventilated bacteriological cabinets are available commercially (S. Blickman, Inc., Weehawken, N. J.; Kewaunee Manufacturing Co., Adrian, Michigan) or can be fabricated from wood, metal or even plastic. Figure 1 shows a typical cabinet constructed of stainless steel. All work is done through the arm-length rubber gloves. An attached pass-through box, equipped interiorly with ultraviolet lamps, is used for inserting or removing items from the cabinet. This cabinet is provided with an exhaust air-filter and an exhaust blower which maintains a reduced pressure of one-half to one in. (water) within the cabinet. Utilities attached to the cabinet include hot and cold water, vacuum, compressed air, gas, drain, a 110 volt AC utility outlet, ultraviolet lamps, and fluorescent lighting.

This cabinet is highly satisfactory for many operations, but it does not prevent possible exposure of the operator to infectious materials removed from the cabinet.

To overcome this problem, a more elaborate and complicated interconnected cabinet system has been developed and has been described in detail by Gremillion (1960). The basic unit of this system is a 34 in. module. A typical general purpose modular system is seen in Figure 2. These cabinets are gastight and have been designed so that all materials leaving the system may be sterilized in the autoclave (seen in the right foreground) by either steam or ethylene oxide gas. Non-autoclavable items may be removed from the cabinets by passing through a disinfectant solution contained in a dunk bath seen in the left foreground. This system can be tailored to the requirements of the user by the incorporation of additional back or bottom mounted units into which are fitted refrigerators, incubators, deep freezers, balances, and sinks.

FIG. 1. Bacteriological safety cabinet. The ultra-violet entry box is seen on the right

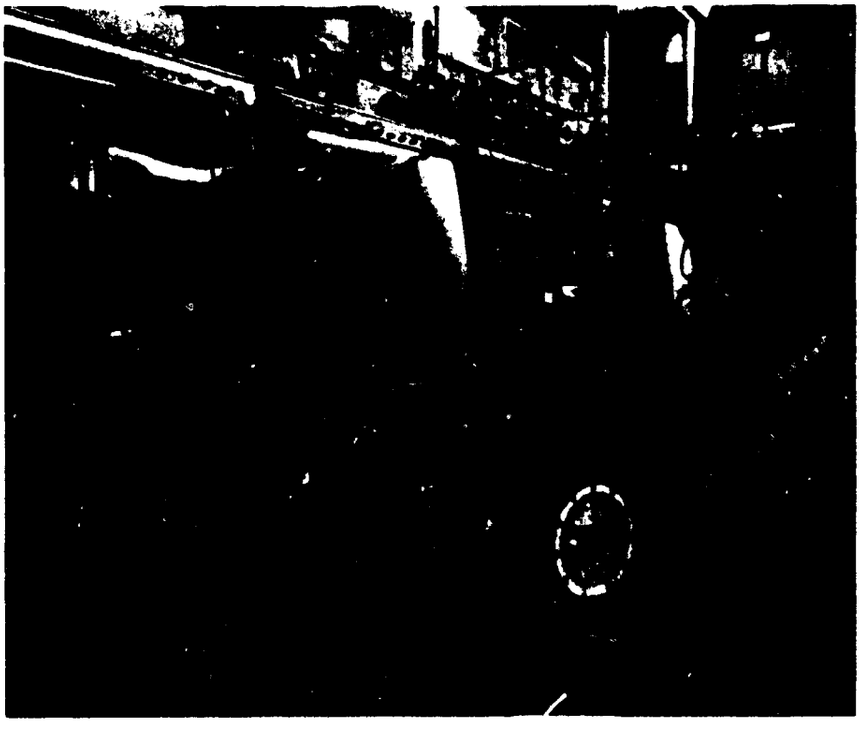

FIG. 2. General-purpose modular hood system. The dunk tank is seen attached to the bottom of the second from left modular unit.

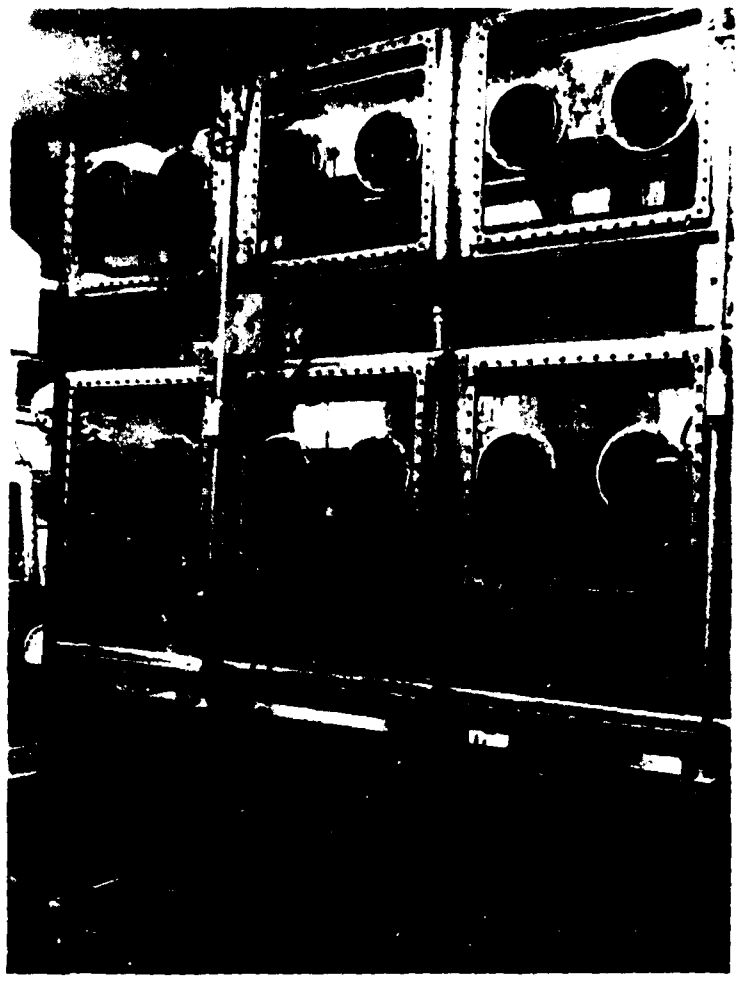

FIG. 3. Animal holding modular units

To protect animal handlers in infectious animal rooms, it may be desirable to house animals in a cabinet system instead of ventilated cages. Such animal holding modules can be added in one or more levels to the basic cabinet system as shown in Figure 3. Various levels are connected to each other by electrically operated elevators (Fig. 4) for the transfer of animals, feed, and equipment. It is within these modular systems that all procedures are carried out with infectious substances. Such procedures include the opening of culture tubes, blending, grinding, inoculating, injecting or necropsying animals, and opening sealed centrifuge cups, or other containers of infectious material suspected of having inner leakage or breakage.

At the conclusion of a particular operation, sterilization of the entire system can best be achieved by vaporizing steam-formaldehyde mixtures (Glick, Gremillion and Bodmer, 1961). For each cubic foot of air-space, one ml of 37% formaldehyde should be vaporized. Most any steam generator can be used as

FIG. 4. Elevator system for animal holding cabinets. The Exposure Tank is on the right and the elevator system is seen on the left of the small push-button control panel in the center of the picture.

the disseminator. Mists of peracetic acid, sprayed from a 2% aqueous solution, also can be used for cabinet sterilization, but precaution must be taken to thoroughly air and wash the cabinet after use of the acid.

Although the bacteriological cabinet provides airtight containment of infectious materials, it cannot serve as a substitute for adequate training and careful techniques.

It is of interest to note that the equipment illustrated is designed and operated for purposes exactly the reverse of that in gnotobiotic methodology. The modular gastight system, under negative pressure, is designed to prevent the escape of organisms from within the system to the outside environment. Gnotobiotic cabinets are maintained under positive pressure to prevent entrance of organisms from the outside environment into the system. Both types of equipment have the identical objective of preventing accidental infections in either the worker or in the gnotobiote.

Granting the essentiality of a cabinet system in infectious disease laboratories, other types of equipment not as spectacular in description but highly contributory to safe operations cannot be overlooked. Pipetting devices are in this cate-

gory. The danger involved in mouth pipetting of pathogenic material is obvious and should never be allowed. A variety of suitable pipetting devices are commercially available (Wedum, 1950). Some of these operate from the laboratory vacuum line. Recommended methods for the use and handling of pipettes as reported by Reitman and Phillips (1950) should be followed.

Another piece of equipment universal in a laboratory is the centrifuge. The biological hazards involved in the operation of this equipment usually consist of an infectious aerosol created by the breaking of glass tubes or the loss of tube stoppers during centrifuging (Reitman and Phillips, 1956). Another hazard is created when infectious fluids are entrapped in the screw threads of screw-capped centrifuge tubes and are thrown out as an aerosol when the centrifuge is operated (Whitwell, Taylor and Oliver, 1957). Safe centrifuging can be accomplished by the use of safety cups or heads. Sealed cups are available to fit either pin-type or conical-type heads on International Company equipment. After centrifuging, all sealed or capped cups should be returned to the bacteriological hood for opening. Table top centrifuges, for which no safety cups are available, should be placed in the safety cabinet during operation.

Another extremely hazardous technique is the grinding or homogenizing of pathogenic material in a high speed blendor. This problem was investigated by Reitman, *et al.* (1953). Out of their studies evolved a sealed container which can be used on any standard Waring blendor to prevent the escape of infectious aerosols during a high speed mixing process. This equipment is particularly important when large numbers of samples of infectious animal tissues must be ground for pathogen isolation or identification.

The frequency of laboratory infections among animal room workers (Wedum, 1956) certainly warrants attention to the procedures and equipment used for holding experimentally infected animals. First and foremost is the need to adequately separate infectious animals from laboratory workers and from other animals. The best isolation obviously is obtained by housing animals individually in sealed cages equipped with filters and ventilated through an air exhaust system. A holding area of individually caged monkeys in ventilated cages is shown in Figure 5. This system has an additional advantage in that separate groups of monkeys infected with various types of infectious agents, for example with tubercle bacilli or *Coccidioides immitis*, can be housed in the same room. It is recognized, however, that in many laboratories the expense of this equipment is prohibitive.

As a practical alternative, germicidal ultraviolet (UV) cage racks have been designed which have effectively reduced the number of air-borne vegetative organisms escaping from contaminated animals (Phillips, *et al.*, 1956). Figure 6 shows a UV rack located along the front wall of an infectious animal holding room. The rack is 5 ft high, 4 ft wide and 22 in. deep, with solid metal shelves. Two 15 w., 18 in., hot cathode UV lamps, with fixtures, are needed for each shelf. Each fixture is equipped with an aluminum reflector to direct the radiation in a

FIG. 5. Ventilated cages for monkeys

band across the tops of the cages. The cages are solid-bottomed and solid-sided to protect the animals from UV radiation. The bottom edge of the lamps is positioned to be level with the top edge of the cages. Studies with this system have shown that the UV barrier prevented cross-contamination from aerosols of vegetative bacteria produced in adjoining cages. Ultraviolet used in this manner was only partly effective against bacterial spores.

Animal workers must wear skin and eye protection when working around UV cage racks. In our laboratories workers wear ventilated personnel hoods as seen in Figure 6 for this protection. Goggles can be worn for short work intervals in this area. It is important that an adequate lamp maintenance program be practiced. The UV lamps must be cleaned frequently and replaced when intensity measurements indicate a 40% loss of output. Intensity meters for UV readings can be purchased from General Electric or the Westinghouse Corporation.

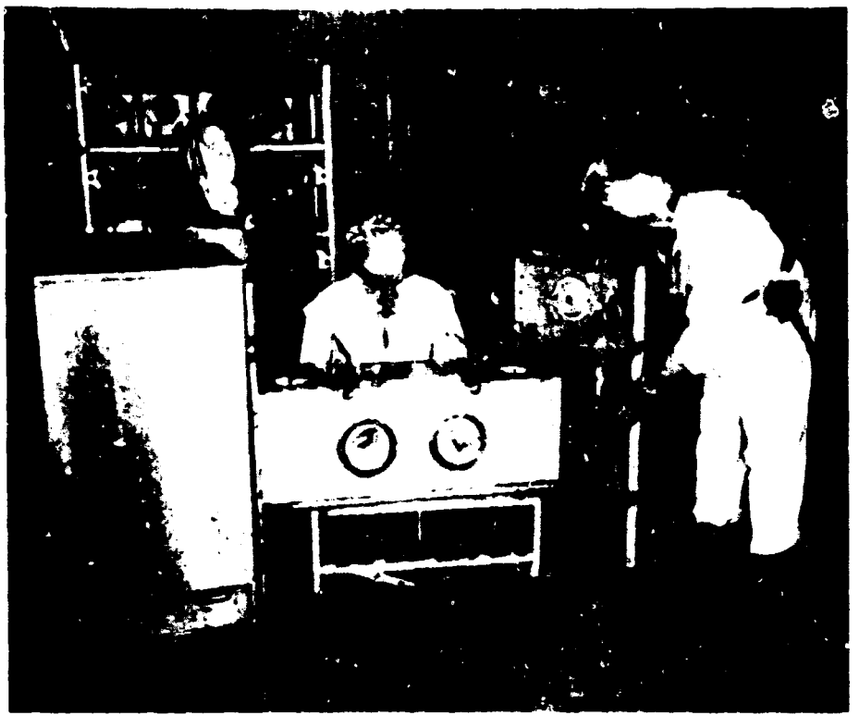

FIG. 6. Ventilated equipment for infectious-animal holding room. Ventilated cage rack outfitted with ultraviolet lights is seen in the background.

An additional feature shown in Figure 6 is a safe method for the transfer of infected monkeys from one ventilated cage to another by means of a transfer tunnel placed between cages. Two guillotine doors, located near the ends of the tunnel, are pulled up and both cage doors are opened. The monkey, in attempting to scamper through the tunnel, can be intrapped in the center by proper maneuvering of the tunnel doors. The sight panel on the top and two glove ports, with attached leather gloves on either side of the tunnel, allow easy restraining and manipulation of the animal. The tunnel also has been useful in work with non-infected monkeys and has resulted in a welcome decrease in the number of monkey bites and pinches in the caretaker group.

To insure continuity in the containment of infectious material during cage changing, the cages are removed from the UV racks and processed in a hood maintained under negative pressure. After transferring an animal to a clean cage, the contaminated cage is placed within a large rectangular "boxcar" which, when full, is trundled into a large double-door autoclave located in the wall between the "contaminated" holding area and the "clean" preparation room (Fig. 7). The time of autoclaving is determined by the contents. After the sterilization period is completed, the boxcar is emptied on the clean side. It then is

FIG. 7. "Box-Car" containing animal cages being placed in double door wall autoclave

returned via the autoclave to the animal holding area for recycling of the process. All animal cages, bedding, carcasses, or other discard materials are passed through these autoclaves for sterilization before they are handled in the clean preparation room.

The disinfection of heat labile materials or delicate electronic equipment is possible by the use of ethylene oxide gas. A steam autoclave can easily be equipped to be used with this gas which is available as a low-pressure mixture with Freon in disposable 16 oz cans. To operate the "gas clave," a vacuum of 14 in. (Hg) is drawn on the chamber and the ethylene oxide gas injected to a positive pressure of 20 psig. A disadvantage in the use of this gas-mixture is the required exposure time of at least six hours. Also, exposed rubber and leather materials must be thoroughly aired after treatment.

For disinfecting single sheets of paper, a UV pass-through chamber installed in a wall separating an "agent" from a "non-agent" area has been described by Phillips and Novak (1956). This apparatus rolls sheets of paper slowly between UV lamps with a radiation dose sufficient to provide inactivation of 99.97% of contaminating bacterial spores. It makes for a very convenient method of passing daily animal records or necropsy protocols for compilation, computation or for immediate transposition into permanent form by clerical personnel.

One of many other uses for germicidal ultraviolet radiation is in the construc-

tion of UV door barriers (Wedum, Hanel and Phillips, 1956). These barriers are constructed by placing UV fixtures in a channel built around a doorway in such a way that a band of radiation screens the opening. It may be very desirable for example, to place these barriers around doorways between infectious animal holding rooms or between rooms housing different species of animals. It should be emphasized, however, that the door barriers are not intended for the decontamination of surfaces, clothing, or equipment, but only for the treatment of air that may pass from one area to another when the doors are opened.

Conclusions

A program for laboratory safety must consist of the firm establishment of clearly stated principles, the education of all personnel in safety operations, the immunization of personnel where applicable, and the proper application of safety equipment. Most of the procedures and equipment for the animal laboratory discussed in this presentation have been proved by actual practice over many years of tests with highly communicable disease agents. Many will counter that the methods and equipment may be too difficult or to expensive to use. However, it was not too long ago that the ultra high speed centrifuge and the electron microscope were considered too expensive and exotic. Today this equipment is found in the majority of laboratories. If one pro-rates the advances made possible by the use of equipment which allows for almost unlimited flexibility and scope of work with infectious agents, it can only be concluded that the real expenses, materially and morally, are the medical and psychic costs incurred when occupational infections occur among laboratory personnel.

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